From molecular mechanisms to gene therapy: novel technologies to tackle ciliopathies using FAM161A-associated retinal degeneration.

Retinitis Pigmentosa (RP), a degenerative disease of the retina with an incidence of 1:4000 worldwide, is a rod-cone dystrophy that slowly evolves into blindness. Among RP cases, RP28 is a ciliopathy affecting uniquely the eye, which has been associated with recessive mutations in the *FAM161A* gene [1-4]. Up-to-now, there is no FAM161A designed therapy to prevent loss of vision or to restore it. Our challenge resides in first describing and understanding the role of FAM161A in RP28 mechanisms, to then offer the appropriate treatment through gene therapy.

In collaboration with the Guichard/Hamel's group at UNIGE, we have recently gained unprecedented understanding on FAM161A precise location by implementing the super-resolution expansion microscopy imaging method (U-ExM). This study conducted in mouse retinal tissue has allowed us to determine some of the disease mechanisms (Mercey et al, *Plos Biology* 2022 [5]). We have in parallel identified promising AAV-FAM161A vectors for gene restoration in preclinical studies in *Fam161A* knockout mice.

The proposed research project aims at (1) deciphering the assembly and biology of the newly-discovered inner scaffold of the connecting cilium, focusing on FAM161A and its interacting partners in human photoreceptors, and (2) pushing forward an impactful gene therapy strategy for FAM161A-associated progressive retinal degeneration. *In vitro* modelling of human photoreceptors and RPE using iPSC-derived retinal organoids technologies will be used to study expression and function of the different isoforms of FAM161A using the Uex-M technology. A FAM161A knockout iPSC cell line will also be generated using CRISPR/Cas9 tools to produce FAM161A-deficient human retinal organoid as a human system to evaluate the efficiency of the AAV-FAM161A. By taking advantage of these innovative technologies, we will further understand FAM161A's role in healthy and diseased conditions and pave the way for a potential human application of the AAV-FAM161A vector.

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